UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION II

DATE: June 5, 2006

SUBJECT: Biological Data Assessment - Sampling and Testing of Material Proposed for Dredging from the San Juan Harbor Section 103, Puerto Rico.

FROM: James A. Ferretti, Aquatic Biologist
Laboratory Branch

TO: Mark Reiss, Environmental Scientist
Dredged Material Management Team

As requested, I have completed the assessment of the biological data collected for the San Juan Harbor Dredging Project. Specific review criteria and comments are included in the attached biological data assessment report.

The findings are listed by major review topics including test type, reference toxicant testing, water quality, and statistical analysis. There were a number of deviations in test design, including organism stocking density, test temperature and test salinity. Also, ammonia purging procedures as amended to our regional guidance was not correctly applied in the tests. Additional information was requested and is highlighted in bold print. Based on findings from the bioaccumulation exposures, it is recommended that the *Macoma nasuta* and *Nereis virens* tests and associated tissue chemistry be repeated. For all other findings, the data user should determine the usability of the data for regulatory decision making.

If you have any questions, please feel free to contact me by phone at 732-321-6728 or via email, ferretti.jim@epa.gov.

Attachment

cc: C. Lynes, DESA-MAB

SAN JUAN HARBOR DREDGE MATERIAL TESTING PROJECT
PPB Environmental Services, Inc, DACW17-03 D-0016, March 2006
Biological Testing QA Review

Testing Laboratory, MACTEC Engineering and Consulting
Report Entitled, "Final Report, Aquatic Toxicity Testing, San Juan Harbor Puerto Rico"

1.0 GENERAL FINDINGS

FINDING 1.1
Page 2, Paragraph 3: A Final Quality Assurance Project Plan had not been approved by USEPA Region 2.

FINDING 1.2
Taxonomic ID for the test organisms was at least three years old. It is recommended that the identifications be performed annually.

2.0 SOLID PHASE TESTING

FINDING 2.1
Page 5, Paragraph 3: *"M. bahia* were six days old at the start of the solid phase test." Based on the raw data at the end of the report, the Mysids used in the solid phase test were three days old. **Please confirm. *M. bahia* for this test must not be more than 5 days old at the start of testing.**

FINDING 2.2
Page 5, Paragraph 5: Five replicates (four were used) must be used for testing the solid phase using *Leptocheirus plumulosus*. Also, the test chamber size should be one liter. A two-quart size was used. **What was the volume (by mass) of sediment and water used to test *L. plumulosus*? What was the resultant depth of sediment in the test chamber?**

FINDING 2.3
Page 5, Paragraph 5: A total of 20 *M. bahia* should be tested per replicate chamber. Only 10 were used in solid phase testing of San Juan Harbor dredge material.

FINDING 2.4
Page 6, Paragraph 2: Appropriate test procedures dealing with porewater and overlying water ammonia were not implemented during solid phase testing of amphipods and mysids. The initial porewater total ammonia was less than 20 ppm and the initial overlying water unionized ammonia was less than 0.6 ppm as unionized ammonia. In accordance with Regional Guidance Manual Protocols, the solid phase tests should have been performed as static non-renewal exposures. The San Juan Harbor solid phase tests were conducted as once daily renewals. **There are no provisions in the RGM for once daily renewals. If ammonia concentrations**

were above the established toxicity thresholds, then the test chambers must be renewed twice daily. However, the solid phase tests for this project should have been conducted without renewals of the overlying water based on the initial porewater concentration for amphipods and overlying water concentrations for mysids.

FINDING 2.5

Leptocheirus plumulosus and *Mysidopsis bahia* used for solid phase testing were not acclimated. Both organisms were used in testing on the same date they arrived at the testing laboratory. A 48-hour acclimation period is recommended to allow the test organisms to acclimate to test temperature and salinity, be fed, and observed for overall health prior to testing.

3.0 SUSPENDED PARTICULATE PHASE (ELUTRIATE TESTING)

FINDING 3.1

Page 4, Paragraph 3: *M. beryllina* are not to be fed during the 96-hour test.

FINDING 3.2

Page 5, Paragraph 2: *A. punctulata* were allowed to be substituted for a suitable bivalve larva because spawnable organisms were not available during the testing of the San Juan dredge material. Only three replicates per sample type were tested. **A minimum of five replicates should be used for any species used in elutriate testing.**

FINDING 3.3

Page 8: For future Region 2 dredge material tests, please do not include data related to parallel control elutriate tests. Also, only a LC or EC50 calculation is required for any elutriate test. T-test comparisons between test and control are not used in any decision making for any of the elutriate tests. The elutriate tests are used to determine the LPC (.01 percent of the LC/EC50 of the most sensitive species tested). These data are used in a dispersion model to predict if the LPC would be exceeded during disposal. The physical effects of the particles do not need to be tested as an interference with use of a control elutriate or at least, not reported in the dredge material data package.

4.0 BIOACCUMULATION TESTING

FINDING 4.1

Page 7, Paragraph 2: The *Macoma nasuta* and *Nereis virens* bioaccumulation tests were performed in the same temperature-controlled room. This would be difficult because the two bioaccumulation tests are conducted using different temperature ranges for each organism. *Macoma nasuta* is tested at 12 - 15 degree's C, while *Nereis virens* are tested at 18 - 22 degrees C. **Were cooling coils or some other means of lowering or raising temperature used with one or both of the test species?**

Page 11, Paragraph 1: For *Macoma nasuta*, " . . . Temperatures were elevated at times due to mechanical failures that were corrected." Based on daily test temperatures from the raw bench sheets, Pages 153-155, temperature for *Macoma nasuta* was outside the prescribed range of 12-

15 degrees C for the entire 28 day test and *Nereis virens* temperatures were outside the prescribed range more than 75 percent of the 28-day exposure based on daily temperature recordings.

FINDING 4.2

A control sample was not tested in the bioaccumulation exposure using *Macoma nasuta* and *Nereis virens*. Control performance is one major test acceptability criterion for the bioaccumulation exposures. The rationale for excluding a control from the test design was not provided in the technical report.

FINDING 4.3

There were other QC issues during bioaccumulation testing of *Nereis virens* and *Macoma nasuta*.

a) There was reduced survival in the reference and two test samples for *Macoma nasuta* (Reference, E-SJ05-A, E-SJ05-B).

E-SJ05-A 28%
E-SJ05-B 42%
Reference - 54%

There is concern when mortality is high in both reference and test samples. The pattern of mortality in this test consisted of 46 - 72 percent mortalities in all three samples tested (control, reference, two test samples). This type of response may be indicative of potential problems with the organisms. Also, since a control sample was not used, additional information regarding organism health, test design, test system, and procedures could not be discerned. USEPA is very cautious in acceptance of data from these types of responses. Survival is not the endpoint in bioaccumulation tests, but tissue chemical residues are measured following a 28-day exposure to the test samples. If there is suspicion of unhealthy or abnormal organisms, this situation could have a direct impact on the uptake, detoxifying, or chemical regulatory systems in the animal. This could lead to either under or over accumulation of target contaminants in the tissue of the test organisms.

Green Book and Regional guidance require the testing laboratory to provide an evaluation of five test acceptability criteria when control survival is less 90 percent: 1) determination if adequate replication exists to maintain statistical power; 2) observation of organism stress; 3) evidence of flow through system contamination; 4) potential control sediment contamination; 5) other quality control problems. While there appears to be enough tissue for analysis, there appear to be other quality control problems which may have directly affected the survival of the organisms. Some of these are listed below:

b) Temperature - *Macoma nasuta* is required to be tested under cooler water temperature conditions (12 - 15 °C). Test temperature was not maintained within this range during the entire 28-day exposure. Actual daily test temperature ranged between 15.3 - 20.3 throughout the 28-day test. Temperatures for *Nereis virens* were outside of the range more than 75 percent of the exposure.

c) Salinity - Prescribed salinity requirements were not met for either *M. nasuta* or *N. virens* on any day throughout the 28-day test.

d) Water Renewals - The 28-day test is required to be performed using **six volume changes per day** in each aquarium. This is usually best accomplished with an automated flow through system.
From the testing notes recorded by MACTEC, test chambers were renewed once manually on eight occasions throughout the 28-day test. The effect of this test design deviation on the uptake mechanisms associated with bioaccumulation is difficult to predict.

e) There was no control sample for *Macoma nasuta* or *Nereis virens*.

Based on these deviations in protocol, particularly temperature excursions and water renewal issues for both *Nereis virens* and *Macoma nasuta*, and the low survival in all samples tested with *Macoma nasuta* (which has the potential to negatively affect uptake mechanisms related to bioaccumulation), it is recommended that the *Macoma nasuta* and *Nereis virens* bioaccumulation tests be repeated. The testing laboratory should put in place procedures to maintain temperature and salinity within prescribed ranges and perform the tests using six volume changes per day per tank as outlined in the test method.

FINDING 4.4
Page 148: Pretest organisms were not depurated for 24 hours in clean seawater prior to freezing

5.0 REFERENCE TOXICANT TESTING

FINDING 5.1
M. bahia and *M. beryllina* reference toxicant tests were conducted for only 48 hours. In accordance with the Green Book and the RGM, 96 hour tests should be performed. If a 96-hour reference toxicant database is not available, a minimum of five 96 hour tests should be performed to establish control chart limits for these organisms for all future Region 2 dredge material evaluations.

6.0 WATER QUALITY

FINDING 6.1
Salinity deviations were noted in six of the seven tests conducted. The RGM provides a set of test condition summary tables for every test organism tested:

Test/Test Organism	Salinity Range Measured During Testing	Test Method Requirement
M. bahia, Elutriate Testing	18.5 - 21 ‰	28 - 32 ‰
M. bahia, Solid Phase	20 - 22 ‰	28 - 32 ‰
M. beryllina, Elutriate Testing	18.5 - 21 ‰	28 - 32 ‰
L. plumulosus, Solid Phase	26 - 29 ‰	18 - 22 ‰
Macoma nasuta, Bioaccumulation	25 - 27 ‰	28 - 32 ‰
Nereis virens, Bioaccumulation	25 - 27 ‰	28 - 32 ‰

FINDING 6.2

Temperature deviations were noted in the following tests:

Test/Test Organism	Temperature Range Measured During Testing	Test Method Requirement
L. plumulosus, Solid Phase	18.4 - 22.1°C	23 - 27 °C
Macoma nasuta, Bioaccumulation	15.3 - 20.3°C	12 - 15 °C
Nereis virens, Bioaccumulation	15.8 - 20.4°C.	18 - 22 °C

7.0 STATISTICAL ANALYSIS

FINDING 7.1

Page 6, Paragraph 3: Statistical comparison of test sediment needs to be compared to the reference sediment only. The control sample is included to assess acceptability of the test method and procedures.

FINDING 7.2

Page 16: **Why was the Kruskal-Wallis test used to determine significant differences between the test and reference samples? Was the ANOVA significant?** Kruskal-Wallis is a non-parametric test used when assumptions of normality and/or homogeneity of variance are not met. A one way unpaired t-test assuming unequal variances may be a more appropriate statistical method to compare test versus reference sediment survival using solid phase test data.

8.0 REFERENCES

USEPA and USACE-NYD, 1992. *Guidance for Performing Tests on Dredged Material Proposed for Ocean Disposal.* USACE-NYD, Water Compliance Section. December 18, 1992.

USEPA, 1994. Memorandum: Southerland, Elizabeth to Mario Del Vicarrio, June 14, 1994. "Recommendations for Conducting Sediment Toxicity Test with Mysidopsis bahia when Ammonia may be Present at Toxicity Levels" (Amended to USEPA and USACE 1992).

USEPA/USACE, 1993. Memorandum: Davies, Tudor, Davis, D.G., Elmore, J.P. to EPA Ocean Dumping Coordinators, Regional Wetlands Coordinators, and USACE Regulatory and Civil Works Elements, December 21, 1993. "Technical Panel Recommendations Concerning Use of Acute Amphipod Tests in Evaluation of Dredged Material; (Amended to USEPA and USACE 1992).

USEPA and USACE, 1991. *Evaluation of Dredged Material Proposed for Ocean Disposal.* Washington, DC. EPA-503/8-91/001.